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Antibiosis resistance against larval cabbage root fly, *Delia radicum*, in wild *Brassica*-species

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Abstract Cabbage root flies (*Delia radicum*) are a major threat to cabbage production in Western Europe and North America. Host plant resistance is the most promising option in controlling cabbage root fly damage. In a no-choice field test, we evaluated 94 accessions belonging to 16 *Brassica*-species for antibiosis resistance against the larvae. Thirteen accessions were selected as putatively resistant, which were subsequently re-tested in the greenhouse. The proportion of eclosed flies was introduced as the main parameter to assess antibiosis in the greenhouse, together with other insect and plant parameters. High levels of antibiosis resistance were identified in *B. fruticulosa* PI663081 and *B. spinescens* BRA2994, with significantly lower proportions of eclosed flies (1 % of the number of eggs used for infestation) compared to other accessions. Both species are difficult to cross with *B. oleracea*. Plants with a high level of antibiosis and medium to high tolerance were found in several accessions of other *Brassica* species (*B. villosa* BRA2922, *B. montana* BRA2950, *B. hilarionis* HRIGU12483, *B. macrocarpa* BRA2944) which are more amenable for crossing with *B. oleracea*. Selection of the most resistant plants belonging to these accessions may yield promising candidates for breeding cabbages resistant to *Delia radicum*.

Keywords Cabbage root maggot · Host plant resistance · Eclosion · *Brassica oleracea* · Insect resistance

Introduction

Cabbage root fly [*Delia radicum* (Linnaeus 1758) (Diptera: Anthomyiidae)] is one of the most damaging pests in cabbage (*Brassica oleracea L.*) production in Western Europe and North America (Dosdall et al. 1994; Finch and Coaker 1969). Female flies lay eggs close to the stem base on the soil surface. Larvae of the root flies feed on the root tissue of the cabbage plants followed by fungal invasion of the wound, which may result in growth retardation or even plant mortality. In temperate zones root fly damage is severe in spring and early summer (Griffiths 1986) when overwintered adult flies oviposit on young plants, whereas in warmer climatic zones the root fly persists the whole year (Joseph and Martinez 2014). In Western Europe

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and North America, economic losses due to root fly damage have been estimated to amount up to $100 million in some years. Furthermore, cabbage root fly infestations cause substantial yield losses in various other *Brassica* crops including broccoli, cauliflower, turnip, and rutabaga (Finch and Ackley 1977).

The threat by cabbage root fly has recently become acute due to the restrictions in the use of chemical insecticides worldwide. Over the last 20 years, farmers extensively applied chemical insecticides to control cabbage root flies. Apart from the fact that the root fly has already developed resistance to many insecticides (Myrand et al. 2015), most of these chemicals are hazardous to the environment and have been banned or are likely to be banned in the near future. For example, the European Union has banned the major insecticide Lindane, a chlorinated hydrocarbon [European Union Regulatory Decision 79/117/EEC (1981) and 304/2003 (00/801)]; in the U.S., increasing restrictions on the use of organophosphate insecticides also led to increased yield loss in cabbage crops due to cabbage root fly (Joseph and Martinez 2014). Furthermore, the lack of effective biological or cultural/physical control methods is an issue. Biological control measures include the use of predators *e.g. Aleochara bipustulata* (Linnaeus 1761) (Coleoptera: Staphylinidae) (Coaker and Williams 1963), parasitoids *Trybliographa rapae* (Westwood 1835) (Hymenoptera: Figitidae) and *Aleochara bilineata* (Gyllenhal 1810) (Coleoptera: Staphylinidae), entomopathogenic nematodes *Steinernema carpocapsae* (Weiser) and *S. feltiae* (Filipjev) (Rhabditida: Steinernematidae) (Georgis et al. 2006) and entomopathogenic fungi e.g. *Metarhizium anisopliae* (Sorokin 1883) and *Beauveria bassiana* (Vuillemin 1912) (Hypocreales: Clavicipitaceae) (Bruck et al. 2005; Chandler and Davidson 2005; Vänninen et al. 1999). However, these methods are either costly or labour intensive, or not effective enough to offer sufficient control of *D. radicum* (Finch 1993; Vänninen et al. 1999; Myrand et al. 2015). Cultural methods such as cover crops are only economical for organic *Brassica* crops sold at higher prices (Finch 1993). Adapting sowing times could avoid root fly infection, but would lead to large reductions in yield (Finch 1993). Other cultural practices such as intercropping (Hummel et al. 2010) and using exclusion fences (Bomford et al. 2000) can reduce crop damage (Dosdall et al. 2000) to a certain extent, yet not sufficiently. Kergunteuil et al. (2015) proposed a push–pull system by intercropping of repellent and trap plants to limit *D. radicum* density, though further investigation on its effectiveness is pending. To cope with the increasing threat by root flies, alternative control methods are urgently needed.

Host plant resistance is the most promising option in controlling insect pests in crops (Broekgaarden et al. 2011; Schoonhoven et al. 2005). Examples can be found in many vegetable crops, *e.g.* host plant resistance in *Lactuca spp.* to the lettuce aphid, *Nasanovia ribisnigri* (Mosely 1841) (Homoptera: Aphididae) was found economically and environmentally effective in controlling this pest (McCreight 2008). The resistance conferred by the *Nr*-locus resulted in reduced performance and feeding rate of aphids (Eenink and Dieleman 1983; ten Broeke et al. 2013). To find host plant resistance, natural variation among wild relatives of crop species can provide good sources (Broekgaarden et al. 2011). Tomato resistance to the whitefly species *Bemisia tabaci* (Gennadius, 1889) (Hemiptera: Aleyrodidae) and *Trialeurodes vaporariorum* (Westwood 1856) (Hemiptera: Aleyrodidae) was found in several wild species and QTLs were identified for reduced oviposition rate (Lucatti et al. 2010, 2013, 2014) and whitefly adult survival (Muigai et al. 2002, 2003; Firdaus et al. 2013; Lucatti et al. 2013). Also, sources of resistance against the Colorado potato beetle *Leptinotarsa decemlineata* (Say, 1824) (Coleoptera: Chrysomelidae) were found among wild relatives of potato (Maharijaya and Vosman 2015). Important resistance to biotic stresses was found among wild *B. oleracea* species (Kole 2011). Regarding insect resistance, Ellis et al. (2000) found germplasm that was resistant to the cabbage aphid *Brevicoryne brassicae* (Linnaeus 1758) (Hemiptera: Aphididae) in *B. villosa* and *B. incana*. Resistance to flea beetles *Phyllotreta cruciferae* (Goeze 1777) was found in *B. incana* (Bodnaryk 1992). In addition, several authors (Bodnaryk 1992; Ramsey and Ellis 1996; Pelgrom et al. 2015) have reported on accessions resistant to cabbage whitefly *Aleyrodes proletella* (Linnaeus 1758) (Hemiptera: Aphididae) among *B. oleracea* var. *capitata* landraces and in the wild species *B. villosa, B. incana, B. montana*, *B. cretica, B. spinosa, B. insularis* and *B. macrocarpa*.

Three resistance mechanisms have been described in the literature on *Delia–Brassica* interactions, antixenosis, antibiosis and tolerance (Painter 1951). Antixenosis, also called non-preference, is based on

morphological and/or chemical characteristics that make a plant unattractive to insects for feeding or oviposition (Painter 1941; Kogan and Ortman 1978; Acquaah 2012). Antibiosis causes adverse effects on insect life history when the insect uses a resistant host–plant variety for food (Painter 1941). Typically, antibiosis increases mortality or reduces the growth and development of insects (Acquaah 2012). This mechanism manifests itself after a host has been attacked and thus affects only the *D. radicum* larvae that feed on the root system. Tolerance refers to the ability of plants to grow and reproduce normally or to repair injury to a marked degree in spite of supporting a population approximately equal to that damaging a susceptible host (Painter 1951). Different from the other two mechanisms, tolerance is independent of the herbivore response, but is an adaptive mechanism for survival of a plant under herbivore pressure (Kogan and Ortman 1978).

A number of studies on the oviposition preferences (antixenosis) of adult *Delia radicum* demonstrated large variation among different crop species and genotypes (Baur et al. 1996; Ellis and Hardman 1975; Ellis et al. 1976, 1979; Kergunteuil et al. 2015). However, this resistance mechanism has not been shown strong enough for preventing economic damage in *B. oleracea* monocultures. Antibiosis resistance to larvae and/or pupae of *Delia spp.* may be the most effective among the three mechanisms mentioned above, and has been reported in wild *Brassica* or other brassicaceous species in several studies. Ellis et al. (1999) screened several *Brassica* species and found that high levels of antibiosis were present in *B. fruticulosa, B. incana, B. villosa* and *B. spinescens,* showing a reduced percentage pupation and high plant survival. They also found that *B. macrocarpa* and *B. villosa* were moderately resistant and all the tested *B. oleracea* accessions and cultivars were highly susceptible. In their study all accessions tested were accepted for oviposition, indicating that antixenosis is not always associated with antibiosis resistance (Finch and Ackley 1977; Wiklund 1975). Jyoti et al. (2001) found antibiosis resistance in *Sinapis alba,* reducing weight and survival of larvae, pupae and adults of *D. radicum*. Resistance to *D. radicum* found in *Sinapis alba* has been successfully transferred into canola (*Brassica napus* L.) as well as *B. rapa* L. and two quantitative trait loci (QTLs) associated with resistance were identified (Ekuere et al. 2005). Malchev et al. (2010) introduced the resistance from canola into rutabaga (*B. napus.* var. *napobrassica*), using marker assisted selection (MAS). In a comparative study of four *B. fruticulosa* and two *B. oleracea* accessions, Felkl et al. (2005) found evidence for antibiosis, as few individuals reared on resistant *B. fruticulosa* accessions developed into pupae that had reduced pupal weight, adult dry weight, and an extended pupal eclosion time. It should be noted that the most susceptible *B. fruticulosa* accession was comparable to the two *B. oleracea* accessions for various damage and insect growth parameters, indicating that within *B. fruticulosa* considerable variation in level of antibiosis resistance against *D. radicum* exists.

In this study we aimed to identify and quantify antibiosis-based resistance to *D. radicum* larvae by screening 94 accessions belonging to 16 species in the genus *Brassica.* For this purpose, no-choice resistance tests were performed in a two-step approach: a field test followed by a greenhouse test. The field test provided an efficient first screening of many accessions, and allowed us to target accessions that possibly possessed resistance. Accessions selected from the field were then subjected to a greenhouse test that allowed a more detailed evaluation of the insect and plant traits important for resistance.

Materials and methods

Plant materials and insect rearing

Seeds of *Brassica* accessions were obtained from different gene banks (Online Resource 1). Plant growing conditions are specified for each experiment below. The colony of *D. radicum* originated from a field at St. Méloir des Ondes (Brittany, France) in 1994 (Pierre et al. 2013), and has been kept as laboratory colony since. The rearing was kept in a climate-controlled cabinet at 22 °C, RH 60 % and a photoperiod of 16 h light/8 h darkness. The method of rearing was based on the description of Neveu and Nenon (1996). The larvae were fed on turnips (*Brassica rapa*) and rutabaga (*Brassica napus*) until pupation. Eclosed adult root flies were kept in gauze cages and were fed on a mixture of sugar, milk powder and yeast in ratio 1:1:1 (Kergunteuil et al. 2015). Tap water was offered in a Petri dish with moist filter paper on top of wet cotton wool. Oviposition was stimulated by placing a

slice of turnip in the cage. The turnip slice was put on top of a moist filter paper in a Petri dish, to prevent desiccation. Eggs were collected around the slice of turnip after approximately 3 h. The eggs were then placed on intact turnips or rutabaga prior to hatching of larvae. At 22 °C, it usually took about 4 days for the larvae to hatch from the eggs.

Field experiment

Resistance screening was carried out in a field (clay soil) near Wageningen, The Netherlands (N51.96, E5.65). Ninety-four accessions of various *Brassica* species (Table 1) were sown in germination trays in May 2012. After germination, plants were transplanted into to Ø 14 cm pots with potting compost (Online Resource 2). Plants were reared in a greenhouse compartment before transplanting into the field. When most of the plants from one accession had 5–6 true leaves, five of each accession were infested with 20 eggs of *D. radicum* per plant (Felkl et al. 2005). Freshly laid or one-day old eggs were used for infestation and placed on the moist surface of the potting compost, close to the stem. One week after infestation the plants were transplanted into the field. Plants were randomized in two blocks and planted at 50 cm distance from each other within a row and 50 or 75 cm between rows. Wilting of leaves, collapse (all leaves wilted and main stem falling over, plants are still green) or death (plant had no green leaves left) were observed twice a week after plants had been transplanted into the field. Plant vigour was scored per accession three weeks after transplanting, using a semi-quantitative scale from 1 to 4, where 1 = all the tested plants are very small and poorly developed, all individuals are wilted or collapsed; 2 = all the tested plants are small, with many individuals wilting, 3 = plants are generally well developed, with two or three of the five plants wilting or small in size; 4 = plants are generally big and well developed, possibly with one or two of the five plants wilting. Subsequently the plants were uprooted and adhering soil was removed from the main and lateral roots. After removing most soil, the roots were rinsed in water to allow observation of the root damage. The numbers of *D. radicum* larvae and pupae were counted. Both the surface of the main roots and the removed soil were carefully checked for larvae or pupae.

Comparing egg and larval infestation

Prior to the greenhouse resistance test, two accessions—*B. oleracea* var. *acephala* (College of Agriculture at Križevci, Croatia, accession A) and *B. oleracea* var *capitata* cv. Christmas Drumhead,

Table 1 List of 16 *Brassica* species and numbers of accessions used in the no-choice field screen, and chromosome numbers for each species

Species	No. of accessions tested	No. haploid chromosomes (n)	Source*
B. balearica	3	16	1
B. bourgeaui	2	9	2
B. cretica	11	9	2
B. drepanensis	2	9	2
B. fruticulosa	22	8	1
B. hilarionis	1	9	2
B. incana	13	9	2
B. insularis	2	9	1
B. macrocarpa	5	9	2
B. maurorum	3	8	1
B. montana	7	9	2
B. oleracea	9	9	2
B. rupestris	3	9	2
B. spinescens	3	8	1
B. sylvestris	1	9	2
B. villosa	7	9	2
Total # accessions	94		

* Source: (1) Prakash et al. (1999); (2) Warwick et al. (2009)

(Centre of Genetic Resources, The Netherlands, accession CGN14080)—were tested using either egg or larval infestation. Seeds were germinated on moist filter paper in a Petri dish. Germinated seeds were then planted into Ø 6 cm Jiffy® pots with potting compost (Online Resource 2). After three weeks, young plants were transplanted into Ø 14 cm pots with the same substrate. After transplanting, plants were grown in the greenhouse (22 ± 2 °C, RH 60 %, photoperiod of 16 h light/8 h darkness). Water was given daily in saucers under the pots, and was taken up by the plants through holes in the bottom of the pots. Nutrient solution (Online Resource 2) was given weekly in the same way as watering. Neither insecticides nor other chemicals were applied to the plants. Egg infestation was done as described for the field infestation. The larval infestation was done by placing neonate larvae (hatched from the egg no longer than one day before) on the moist potting soil surface, close to the stem. The larvae were observed crawling into the soil. Twenty eggs or larvae were inoculated on each plant. Eight plants per accession were infested by each method.

Greenhouse resistance test

Based on the field test results obtained in 2012, accessions with on average less than one *D. radicum* larva or pupa per plant were considered putatively resistant (Online Resource 1). From each species, at least one accession was selected and subjected to a detailed resistance test in the greenhouse. The accessions were selected using the following criteria (compared to all the other accessions of the same species): 1. The lowest number of plants showing wilting, collapse or death; 2. Highest plant vigour; 3. Lowest number of negative remarks on plant development (*e.g.* 'extremely small plant').

In mid-January 2013, 16 accessions were sown, including 13 accessions of wild *Brassica* species that were selected as putatively resistant, and three *B. oleracea* cultivars/accessions. *Brassica oleracea* BOL2010-0437 (*B. oleracea* Rapid Cycler) and *B. oleracea* CGN14080 (*B. oleracea* cv. Christmas Drumhead) were used as susceptible controls. *Brassica oleracea* var. *acephala* Accession A was not tested in the field, but was included because a successful cross between *B. fruticulosa* and *B. oleracea* var. *acephala* Accession A had been made (Pelgrom et al. 2015). Seed germination and plant growing conditions were the same as described in the experiment comparing larval and egg infestation.

Twelve seedlings per accession were germinated. Out of these seedlings ten were randomly picked and potted, and were subsequently tested. The infestation was done as described in the no-choice field experiment. Wilting of plants was scored starting one week after infestation. Three weeks after infestation, each plant was enclosed in a textile sleeve to entrap eclosing flies. Adult flies eclosed from each plant were collected daily and counted until no more flies eclosed during seven days. Flies collected per plant were stored at −20 °C and transferred individually into Eppendorf tubes for drying at 50 °C for two days. Dry weight of the flies was measured using a Sartorius® CP2P-F Micro Balance.

At the end of the experiment, plants were taken out of the pots and the roots were cleaned with water. Plant shoot vigour was scored semi-quantitatively on a scale from 0 to 5, where 0 = dead plant with no green leaf left; 1 = 1/5 of the leaves and stems are green, plant collapsed; 2 = 2/5 of the leaves and stems are green, plant collapsed, 3 = 3/5 leaves and stems are green, main stem stands up-right; 4 = 4/5 leaves and stems are green, plant stands up-right with a few leaves collapsed/wilted; 5 = plant is well developed, all the leaves and stems are green/only a few leaves are partly wilted/yellow, plant stands up-right. Root vigour was also scored semi-quantitatively on a scale from 0 to 2, where 0 = no main root left, fine roots hardly found; 1 = small main roots and several fine roots; 2 = strong main roots and numerous fine roots. Root damage was scored on a semi-quantitative scale modified after Dosdall et al. (1994) where 0 = no root damage; 1 = small feeding channels on the root comprising less than 10 % of the root surface area; 2 = 11–25 %; 3 = 26–50 %; 4 = 51–75 % and 5 = 76–100 % of the tap root surface area; 6 = root is damaged deep into its core tissues and only a small core of the tap root is left. It should be noted that a root surface damage score of 5 did not necessarily indicate a dead plant, as a large part of the root core is still functional and some plants may regrow new roots, while a root damage score of 6 often resulted in a dead plant. Finally plants were oven-dried at 70 °C for 2 days and the dry weight of each plant was measured.

Statistical analysis

For the field experiment, the fraction of pupae or larvae retrieved was transformed as y = arcsin (sqrt(x)) and subjected to Analysis of Variance (ANOVA). Accession means were calculated for number of larvae and pupae, plant vigour, number of days until wilting and number of days until collapse. The mean number of days until wilting or collapse was calculated based on the actual number of plants that wilted or collapsed. For the greenhouse experiment, fraction of total flies eclosed, average fly dry weight, days before the first fly eclosion, eclosion period, *i.e.* the number of days during which flies eclosed (from the first till the last fly eclosing), root damage, shoot vigour, root vigour, plant dry weight, and days before plant wilting were analysed by ANOVA and means separated using Fisher's least significant difference (LSD). Fly eclosion was expressed as fraction of eclosed flies out of number of infested eggs. This fraction was transformed into arcsin (sqrt(x)) to stabilize variance. To analyse the effect of different infestation methods a two-way ANOVA was used, with two factors—the effect of infestation method and the effect of accession. For the larva and egg infestation trial parameters analysed included number of flies eclosed, the number of days before the first fly eclosed, and eclosion period. A log transformation was applied to eclosion period in order to stabilize variance. The other parameters showed normal distribution. For statistical calculations IBM SPSS Statistics for Windows (Released 2011, Version 20.0. Armonk, NY: IBM Corp.) and GenStat (17th edition, VSN International Ltd, United Kingdom) were used.

Results

Field experiment

The aim of the field experiment was to identify accessions putatively resistant to *D. radicum*. Out of the 94 accessions tested, which belonged to 16 *Brassica* species (Table 1), 36 had zero *D. radicum* and 25 accessions had on average less than one *D. radicum* pupa or larva per plant (Online Resource 1). Accessions on which no *D. radicum* larvae or pupae were found belonged to several wild species, including the biennial species *B. balearica, B. cretica, B. drepanensis, B. hilarionis, B. incana, B. macrocarpa, B. villosa*, and the early-flowering annual species *B. fruticulosa* and *B. spinescens*. Fourteen out of a total of 22 accessions of *B. fruticulosa* were free of *D. radicum* larvae or pupae. Among all 94 accessions, in 52 at least one of the five plants wilted, in 10 accessions at least one plant collapsed and in 4 accessions at least one plant died. The proportions of wilted, collapsed, and dead plants within each accession are shown in Online Resource 1. The number of retrieved *D. radicum* larvae or pupae did not show significant correlations with either plant vigour, nor the number of days until plant wilting/collapse.

Comparing egg and larval infestation

Comparison of the infestation methods in the greenhouse showed no significant differences in the number of flies eclosed and in the length of the eclosion period (Table 2). Also, no interaction effects were detected for these two parameters. The effect of infestation method and the interaction effect infestation methods with accession were significant for the number of days before the first fly eclosion. The means of the number of flies eclosed from plants infested with eggs or larvae, are given in Online resource 3.

Greenhouse resistance test

From the field experiment, the 13 most resistant accessions were selected for confirmation under greenhouse conditions. Significantly lower proportions of root fly adults eclosed on the accessions *B. fruticulosa* PI663081 and *B. spinescens* BRA2994 (mean 1 %) than on the other accessions (Table 3). A moderate proportion of eclosed flies was found on *B. fruticulosa* BRA1727 and *B. hilarionis* HRI-GRU12483, yet they were not significantly different from a few other accessions. Within several accessions a few individual plants were found from which no flies eclosed (Table 3), although the mean number of flies of these accessions was high. For example, within *B. macrocarpa* BRA2944 and *B. villosa* BRA2922 respectively, one plant was found free of *D. radicum*. A large variation in mean fly dry weight (1.3–2.9 mg) was observed among the accessions (Table 3). Flies with the smallest dry weight developed on *B. spinescens* BRA2994 and *B. fruticulosa* BRA1727. Flies eclosed on *B. fruticulosa* PI663081, *B. hilarionis*

Table 2 Two-way ANOVA results to assess the effect of infestation methods (larvae or eggs) and accession (*B. oleracea* CGN14082 and *B. oleracea* CGN14082) on fly survival and eclosion

Trait	*P* value infestation method effect	P value accession effect	P value interaction effect
Number of flies eclosed	0.706	0.409	0.711
Days before eclosion[a]	<0.0001	0.106	0.014
Eclosion period[b]	0.340	0.082	0.556

Twenty eggs or larvae, both were one-day old, were used to infest each plant. Eight plants per accession were used

[a] Number of days of *D. radicum* development from the day of infestation until eclosion of the first fly

[b] Number of days between the first and the last fly eclosing

Table 3 Accession means of insect characteristics and the number of individual plants (out of 10 tested plants) with zero *D. radicum*, greenhouse resistance test

Species	Accession	Insect trait				Number of individual plants with zero *D. radicum*
		Total flies eclosed (fraction)[a]	Fly dry weight (mg)	Days before first fly eclosion	Eclosion period	
B. bourgeaui	BRA2848	0.32cd[b]	2.9ef	32.3abc	3.5bc	1
B. cretica	PI662588	0.26bcd	2.5de	32.6abcd	4.4cd	1
B. drepanensis	BRA3093	0.30bcd	2.9ef	33.0bcdef	3.7bc	2
B. fruticulosa	**PI663081**[c]	0.01a	1.7ab	32.5abcd	1.2a	7
B. fruticulosa	BRA1727	0.12b	1.3a	31.3a	3.7bc	4
B. hilarionis	HRIGU12483	0.17bc	2.0bc	32.2abc	1.9ab	2
B. incana	BRA2856	0.34cd	2.7ef	32.9abcde	4.5cd	0
B. incana	PI435898	0.43d	2.9ef	32.5abcd	4.9cd	0
B. macrocarpa	O-502	0.30bcd	2.9ef	33.7cdefg	4.4cd	0
B. macrocarpa	BRA2944	0.33cd	3.1f	33.0bcdef	4.3cd	1
B. montana	BRA2950	0.32cd	2.6e	34.4efg	4.8cd	1
B. spinescens	**BRA2994**[c]	0.01a	1.3a	34.6fg	3.9c	9
B. villosa	BRA2922	0.27bcd	2.1cd	35.0g	4.7cd	1
B. oleracea	Accession A	0.18bc	2.6e	34.1defg	4.0cd	3
B. oleracea	BOL2010-0437	0.36cd	2.6de	32.7abcde	4.9cd	0
B. oleracea	CGN14080	0.44d	2.7ef	31.8ab	5.8d	0

[a] Arcsin(sqrt(fraction)) of number of eclosed flies used for ANOVA was back-transformed to fraction eclosed flies. Twenty eggs were infested per plant

[b] Means followed by the same letter are not significantly different ($P > 0.05$) according to Fisher's Least Significant Difference (LSD) test

[c] *Brassica fruticulosa* PI663081 and *B. spinescens* BRA2994 have been typeset in bold to indicate that the fraction of total flies eclosed differed significantly from all other accessions

HRIGU12483 and *B. villosa* BRA2922 showed intermediate dry weight (1.7–2.1 mg). On the remaining accessions flies had a higher dry weight (>2.2 mg). The reference accessions of *B. oleracea* BOL2010-0437 (*B. oleracea* Rapid Cycler) and *B. oleracea* CGN14080 (*B. oleracea* cv. Christmas Drumhead), produced the highest proportion of eclosed flies among all tested accessions (36 and 44 %), with relatively high average fly dry weight (2.6–2.7 mg). Accessions of several other *Brassica* species had statistically similar values. *Brassica oleracea* acephala Accession A produced a low proportion of eclosed flies (18 %),

but 80 % of the plants wilted. The number of days until fly eclosion ranged from 31.3 days (*B. fruticulosa* BRA1727) to 35 (*B. villosa* BRA2922) and the fly eclosion period from 1.2 to 5.8 days (Table 3).

The root damage score shows that more than 25 % root surface area was damaged in all the accessions (Table 4). The roots of *B. oleracea* BOL2010-0437 were damaged most seriously, nearly all the tap roots were consumed. The two accessions on which the lowest proportion of flies eclosed, *B. fruticulosa* PI663081 and *B. spinescens* BRA2994, also showed high root damage score. The lowest root damage scores were found on accession *B. bourgeaui* BRA2848 (3.1) and on *B. oleracea* Accession A (3.2).

The accessions on which it took the longest time before the plants started wilting were *B. fruticulosa* BRA1727, *B. villosa* BRA2922 and *B. incana* BRA2856. Among these three, the proportion of wilted plants was high on *B. incana* BRA2856 and low on the other two. *Brassica fruticulosa* PI663081 and *B. macrocarpa* BRA2944 also belonged to the group with the longest period until wilting, but this was not significantly different from several other accessions. All 16 accessions tested in the greenhouse experiment showed symptoms of wilting. Out of these, only seven accessions showed symptoms of wilting in the field experiment, with a maximum of two plants out of five tested. The other eight accessions did not show wilting in the field. Unlike most other tested accessions, the reference cultivar *B. oleracea* CGN14080 supported more root flies in the field than in the greenhouse test (Online Resource 1, Table 3).

Table 4 Accession means of plant characteristics, greenhouse resistance test

Species	Accession	Plant trait					
		Root damage[a]	Shoot vigour[b]	Root vigour[c]	Plant dry weight (g)	Days until wilting	% wilted plants
B. bourgeaui	BRA2848	3.1 a[d]	4.3ef	1.8def	9.5ef	14abc	100
B. cretica	PI662588	5.3cde	2.3ab	0.6a	4.6abc	12a	100
B. drepanensis	BRA3093	4.4bc	3.3bcde	1.5bcde	6.4bcd	14abc	50
B. fruticulosa	**PI663081**[e]	5.4cde	4.8f	1.9ef	6.2abcd	18ef	50
B. fruticulosa	BRA1727	5.6de	4.3ef	1.9ef	7.2cde	20f	30
B. hilarionis	HRIGU12483	5 bcde	2.1a	0.9ab	3.3a	14abc	44
B. incana	BRA2856	4.7bcd	3.9def	1.7cde	7.4cde	19f	90
B. incana	PI435898	4.6bcd	3 abcd	1.1abcd	7.6de	15abc	80
B. macrocarpa	O-502	5.3cde	3.8cdef	1.5bcde	5.5abcd	16bcde	75
B. macrocarpa	BRA2944	5 bcde	3.3bcde	1.2abcde	6.1abcd	18def	20
B. montana	BRA2950	5.3cde	3.3bcde	1.5bcde	6.3bcd	16cde	60
B. spinescens	**BRA2994**[e]	4.5bc	3.7cde	1abc	4.3ab	16bcde	70
B. villosa	BRA2922	4.6bc	4.2ef	2.4f	7.2cde	20f	50
B. oleracea	Accession A	3.2a	4def	1.9ef	11.6f	17cde	80
B. oleracea	BOL2010-0437	6e	2.7abc	0.6a	5.3abcd	15bcd	70
B. oleracea	CGN14080	4.1ab	3.9def	1.4bcde	9.7ef	14ab	100

[a] Root damage was scored on a modified semi-quantitative scale (Dosdall et al. 1994) where 0 = no root damage; 1 = small feeding channels on the root comprising less than 10 % of the root surface area; 2 = 11–25 %; 3 = 26–50 %; 4 = 51–75 % and 5 = 76–100 % of the tap root surface area; 6 = Root is damaged deeply and only a small core of the tap root left

[b] Plant shoot vigour was scored semi-quantitatively on a scale from 0 to 5 (very poor/dead–well developed, see Materials and Methods section Greenhouse resistance test.)

[c] Root vigour was scored semi-quantitatively on a scale from 0 to 2 (no root/dead – good root system, see Materials and Methods section Greenhouse resistance test.)

[d] Means followed by the same letter are not significantly different ($P > 0.05$) according to Fisher's least significant difference (LSD) test

[e] *Brassica fruticulosa* PI663081 and *B. spinescens* BRA2994 have been typeset in bold to indicate that the fraction of total flies eclosed differed significantly from all other accessions

Correlations between traits were detected using accession means and individual plant data (Table 5, Online resource 4). Among insect traits, a few significant correlations were found. Based on the accession means, the number of flies eclosed showed a strong positive correlation with fly dry weight (Table 5; Fig. 1) and with eclosion period. These two correlations were also significant when calculated based on individual plant data (Online resource 4). Individual plant data also showed a negative correlation between the number of flies eclosed and the days before the first fly eclosion (Online resource 4).

Among plant traits, several significant correlations were found. The accession means and individual plant data showed that shoot vigour, root vigour and plant dry weight were positively correlated with each other; plant dry weight was negatively correlated with root damage. For individual plant data, root damage was also negatively correlated with shoot vigour and root vigour. The number of days until wilting was positively correlated with both shoot and root vigour in both accession means and individual plant data. Besides, individual plant data also showed that days until wilting was correlated positively with plant dry weight. The percentage of wilted plants per accession showed no significant correlation with any other trait.

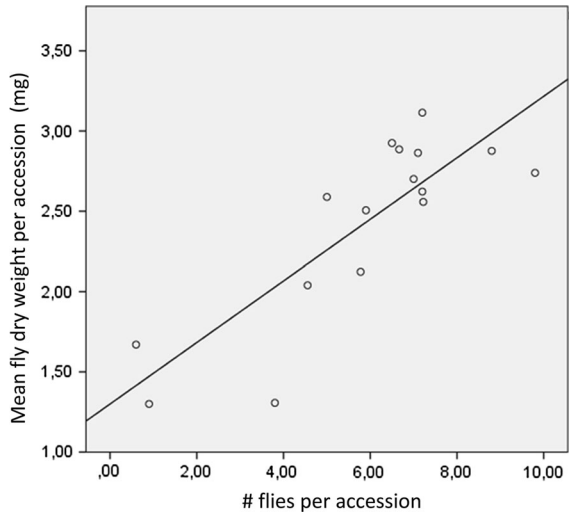

Fig. 1 Scatter plot of the number of flies retrieved per accession and mean fly dry weight per accession (Spearman's rho 0.71, $P < 0.0001$)

Some insect traits showed correlations with the plant traits based on individual plant data. The number of flies eclosed was negatively correlated with shoot and root vigour and plant dry weight. The number of flies was positively correlated with the average root damage. The number of days until the first fly eclosion was positively correlated with root vigour, plant dry

Table 5 Spearman's correlation coefficient between parameters of the greenhouse test, based on accession means

	Fly dry weight	Days before fly eclosion	Eclosion period	Root damage	Shoot vigour	Root vigour	Plant dry weight	Days before wilting	% Wilted plants
Fraction of flies eclosed	0.71**	−0.05	0.75**	−0.13	−0.38	−0.36	0.31	−0.34	0.32
Fly dry weight		0.05	0.31	−0.33	−0.10	−0.06	0.36	−0.23	0.28
Days before first fly eclosion			0.23	−0.16	−0.06	0.11	−0.12	0.33	−0.10
Days of fly eclosion				0.05	−0.29	−0.27	0.23	−0.03	0.38
Root damage					−0.25	−0.23	−0.57*	0.23	−0.41
Shoot vigour						0.88**	0.57*	0.55*	0.04
Root vigour							0.612*	0.64**	−0.14
Plant dry weight								0.18	0.40
Days before wilting									−0.47

* Correlation is significant at the 0.05 level (2-tailed)

** Correlation is significant at the 0.01 level (2-tailed)

N = 16

weight, and days until wilting. Neither the number of days until wilting nor the percentage wilted plants showed a significant correlation with the proportion of eclosed flies.

No correlation was detected between the wilting-related parameters in the field and any parameters in the greenhouse (Online resource 5), although the accessions that produced the lowest proportion of eclosed flies in the greenhouse test showed no wilting in the field. However, some of the accessions without wilting symptoms in the field did produce flies in the greenhouse (Online Resource 1).

Discussion

Resistance screening methodology

Egg versus larval infestation

Egg infestation is easy to handle in practice and is less labour-intensive than larval infestation. Recent studies showed that herbivore eggs affect plant direct and indirect defence (Hilker and Fatouros 2015). Although in nature *D. radicum* lays its eggs not directly in contact with the plant (Zohren 1968 cited in Schoonhoven et al. 2005), root exudates contain secondary metabolites (Schreiner et al. 2011) that could potentially influence egg development and survival. Therefore we tested both egg and larval infestation using two accessions. We found no significant difference in the proportion of adult flies eclosing between the two infestation methods. Since egg infestation did not differ from larval infestation for antibiosis resistance screening under greenhouse conditions, egg infestation was chosen as the standard method.

Parameters for resistance screens

The proportions of successfully developing larvae and pupae are commonly used to assess the level of antibiosis resistance to *D. radicum* (Finch and Ackley 1977; Ellis et al. 1999; Jyoti et al. 2001; Felkl et al. 2005). In the field experiment we collected larvae and pupae from the soil around the root system. In the greenhouse experiment we employed a new parameter—the proportion of eclosed flies—for antibiosis assessment. Instead of uprooting the plants and collecting larvae or pupae, we allowed the pupae staying underground undisturbed until adult flies eclosed. There are several advantages of using this method compared to counting larvae/pupae. Firstly, it includes the possible effect of the plant on pupal mortality/development. Some researchers collected pupae and evaluated fly eclosion under artificial conditions (Jyoti et al. 2001; Felkl et al. 2005), which excluded the possible effect of the plant root environment on pupal survival. Felkl et al. (2005) reported the fraction of flies that eclosed from pupae to range from 0.82 to 0.99 for the four tested *B. fruticulosa* accessions, and 0.85 for *B. oleracea* BOL2010-0437. Secondly, it allows to assess the development of *D. radicum* more accurately. The pupal stage lasts 3 weeks, thus it is hard to estimate pupal age when pupae are found. By using eclosed flies we can obtain precise data on the days elapsed before eclosion, and the eclosion period. Thirdly, it avoids the risk of overlooking larvae or pupae in the soil and the labour involved in searching for them. Eclosed flies were trapped in sleeves and thus were easy to collect. Instead of measuring the weight of pupae and larvae (Jyoti et al. 2001), dry weight of adult flies was measured in our greenhouse experiment as another parameter for insect development and growth. Although not conducted in this research, using eclosed flies also provides the possibility to assess the fecundity of females allowing to predict population development of *D. radicum* on a particular accession.

The fraction of eclosed flies developed from the 20 inoculated eggs per plant ranged from 0.01 to 0.44, which is comparable to the survival rate from no-choice resistance screens in previous studies. In the study of Felkl et al. (2005), the highest *D. radicum* pupae recovery was ~15 % on the cauliflower *B. oleracea* var. *botrytis* cv. Fremont. In the study of Jyoti et al. (2001), the highest larval survival was ~31 % on the cauliflower *B. oleracea* var. *botrytis* cv. Amazing, and the highest pupal recovery rate of ~22 % was found on the broccoli *B. oleracea* var. *italica* cv. Green Comet. In the study of Finch and Ackley (1977), 18 and 38 % of inoculated eggs produced pupae on *B. oleracea* when infested at different plant ages. The papers cited above used 10–20 eggs for infestation. It is possible that infestation with a higher number of eggs would give stronger effects on the plant phenotype. However, it should be noted that larval feeding damage was observed on

almost all the accessions, including on the most resistant ones. A higher infestation pressure might lead to early plant mortality due to a quick consumption of the root system, as well as the loss of the part of the insects due to severe competition. To avoid this, an infestation with 20 eggs was used.

Parameters of plant performance are vital for breeders and have been used in the literature as a tool for selecting resistant plant material. Root damage is an important parameter for antibiosis, especially in determining whether plants supported early larval development yet not sufficiently to develop into pupae. Root damage and root vigour give a good indication of the extent of larval damage and plant performance respectively. Plant vigour and plant-morphological traits show high variation within an accession. Plants with higher root and shoot vigour might have a certain level of tolerance to the insect damage (Painter 1941; Acquaah 2012).

Wilting and collapse are typical symptoms of *Brassica* plants damaged by cabbage root fly larvae. Jensen et al. (2002) studied the resistance of 14 accessions of *B. fruticulosa* by evaluating the number of days before plant wilting or collapse after root fly infestation and plant survival, and found *B. fruticulosa* in general survived more days without collapse compared to *B. oleracea*. Ellis et al. (1999) reported high (*B. fruticulosa, B. incana, B. spinescens*) and moderate (*B. macrocarpa* and *B. villosa*) levels of antibiosis resistance, using the number of pupae together with the parameters "percentage of damaged plants per accession", "the mean time to collapse", and "the mean time to 50 % collapse". In our experiments parameters related to wilting proved to be unreliable as criteria for quantifying resistance. Firstly, plants of different species/accessions vary in leaf morphology, including the toughness of leaves, which may influence the phenotype of wilting under the same root fly pressure. *Brassica spinescens* and *B. fruticulosa* both have a small total leaf area which may reduce transpiration rate and wilting (assuming equal number of stomata per unit leaf surface of all accessions), making it questionable to compare these species with other species with a larger leaf area. Secondly, the symptoms of wilting over time also vary between accessions. For example, *B. oleracea* BOL2010-0437 typically showed early wilting of the old and young leaves which was soon followed by the collapse of the entire plant including wilting of the flowers. In contrast, *B. fruticulosa* and *B. spinescens* usually showed wilting of a few old leaves subsequently resulting in yellowing and loss of these leaves, while wilting of young leaves and plant collapse was not observed. Thirdly, wilting symptoms can be caused by other reasons such as drought as well as other pests and pathogens in the field. Thus "wilting" solely cannot be used as a criterion for antibiosis resistance among different species/accessions, although it can serve as a measure of tolerance.

The insect parameters that we used primarily related to antibiosis, as they directly measure the mortality, growth and development of the insect. The plant parameters focus on plant growth and tolerance during or after larval feeding. In general it was remarkable that no significant correlation was found between the plant and insect related parameters based on accession means. Yet based on individual plant data, some significant correlations were found (Online resource 4), for example the fraction of eclosed flies correlated with all the other insect and plant traits except with days until plant wilting. Variation within accessions may explain why correlations among accession means were not significant. Root vigour and shoot vigour showed strong positive correlation suggesting that they measure the same underlying trait relevant to rootfly tolerance. Wilting can be the result of root fly damage, but we found no correlation between wilting and insect parameters. Again we conclude that wilting is more relevant to tolerance, as accessions with high level of tolerance could show delayed wilting or a low proportion of wilted plants.

For future antibiosis resistance screenings, we propose a methodology including the scoring of the number of eclosed *D. radicum* (preferably adult, or larvae/pupae), root damage level, and the shoot or root vigour. Insect survival should be the key parameter, as it determines the growth of the insect population. Quantifying the fraction of eclosed flies provides more informative data and is practical when testing plants in pots. To predict population development of *D. radicum* on a particular accession, it would be useful to sex the eclosed flies, and collect the fecundity data of the females. Root damage provides direct evidence of larval feeding. Both shoot and root vigour provide valuable information on plant tolerance to root fly attack. Both field and greenhouse screens are essential in identifying antibiosis among wild *Brassica* species. No-choice field screening is a realistic, economical

and efficient method for identifying potentially resistant candidates among many accessions. To avoid missing larvae/pupae and reduce the effect of predators, a field experiment could be replaced by a potted plant experiment outdoors, using big pots filled with substrate and sand. A greenhouse test is important to confirm the resistance and susceptibility of plants in a controlled environment. One issue that also should be noted is the fact that we used a *D. radicum* population that was maintained under laboratory conditions for many years. This may have resulted in selection of a genotype best adapted to these conditions, which is probably different from the original field collection. For a more thorough confirmation of the selected accessions, a test using more *D. radicum* populations is highly recommended.

Comparison of field and greenhouse experiments

A no-choice approach was applied in the field experiment. This approach differs from most literature on *D. radicum* resistance in which no-choice tests were often conducted in the greenhouse, complementing choice tests in the field (Finch and Ackley 1977; Ellis et al. 1999; Jyoti et al. 2001). Given a choice, female root flies prefer to oviposit on some *B. oleracea* accessions over others (Dosdall et al. 1994; Finch and Ackley 1977; Ellis et al. 1999; Jyoti et al. 2001), but in large monoculture fields no such choice is possible. No-choice tests through egg infestation in the field ensured that the selection of resistance was based on antibiosis, not antixenosis. Of course many factors can influence the results in the field, e.g. wild females might lay eggs on pre-infested plants, leading to higher number of larvae/pupae on some accessions. In this case differences in developmental stages may be used to identify plants infested by wild *D. radicum* from the pre-infested *D. radicum* during evaluation, if desired. As we focussed on plants and accessions with the lowest number of insects the ones with more larvae and pupae were not selected.

The testing environment influenced the results considerably, particularly in the number of insects retrieved and in wilting. Greenhouse conditions seemed to be optimal for insect growth as the temperature and moisture level was similar to those prevailing in the *D. radicum* rearing. Most of the accessions produced higher proportions of eclosed flies in the greenhouse experiment than the proportions of larvae and pupae they produced in the field experiment. Several accessions on which zero *D. radicum* larvae or pupae were found in the field experiment showed varying levels of susceptibility in the greenhouse test. Also, more plants showed wilting in the greenhouse than in the field. In the field, plants are exposed to a more complex biotic and abiotic environment that potentially influences the phenotype. Natural enemies of underground herbivores can be attracted (Van Tol et al. 2001; Rasmann et al. 2005), as some accessions might be more efficient in this type of indirect defence by recruiting more natural enemies. Plant root morphology may also differ considerably between field and greenhouse conditions, possibly affecting the survival and the development of the larvae (Felkl et al. 2005). In addition, differences in soil conditions might also influence herbivore-associated organisms, which subsequently affect insect physiology and the plant phenotype (Zhu et al. 2014). Moreover, environmental conditions may have a large effect on tolerance, resulting in differences in plant performance (Painter 1951).

Inoculation under greenhouse conditions was found suitable to determine cruciferous hosts of *D. radicum* (Finch and Ackley 1977). With strong influence of environment, the choice of appropriate reference accessions becomes important when plants are tested under both field and greenhouse conditions. The *B. oleracea* CGN14080 showed stable performance under both field and greenhouse conditions, in contrast to *B. oleracea* BOL2010-0437 that showed a more variable result. No larvae or pupae were retrieved from *B. oleracea* BOL2010-0437 in the field test. In our greenhouse experiment, *B. oleracea* BOL2010-0437 exhibited the highest number of flies, the highest root damage score and the highest proportion of wilted plants, indicating a combination of low antibiosis and low tolerance (Felkl et al. 2005). In other studies, moderate to low number of *D. radicum* larvae or pupae were found on *B. oleracea* BOL2010-0437 (Ellis et al. 1999; Jyoti et al. 2001; Jensen et al. 2002; Felkl et al. 2005). Similar to *B. oleracea* BOL2010-0437, the other early-flowering accessions of *B. fruticulosa* and *B. spinescens* showed relatively high root damage scores. This is partly due to their small root system. As is showed by Felkl et al. (2005), plants with long main roots and a large number of lateral roots often had higher tolerance to root fly damage. The biennial *B. oleracea* CGN14080, although it showed low

antibiosis and supported a high number of flies, was relatively high in tolerance thus the root damage score was lower than for *B. oleracea* BOL2010-0437. The larger root system has probably contributed to the high tolerance of CGN14080.

Selection of resistant plants

Accessions with the strongest antibiosis resistance belong to *B. fruticulosa* and *B. spinescens.* In several studies, antibiosis resistance was identified in accessions of *B. fruticulosa* (Ellis et al. 1999; Jensen et al. 2002; Felkl et al. 2005). In their studies *B. fruticulosa* BRA1039 was found to be resistant, and in our field test this accession showed 1 % survival of infested *D. radicum* on average, thus was left out of our greenhouse confirmation test as we selected only the accessions that did not support any *D. radicum* survival. Some *B. fruticulosa* accessions have shown resistance towards several other insects as well, including the aphid *B. brassicae*, the cabbage whitefly *A. proletella*, and the green peach aphid *Myzus persicae* (Sulzer, 1776) (Hemiptera: Aphididae) (Singh et al. 1994; Ellis et al. 1996, 2000; Pelgrom et al. 2015). Ellis et al. (1999) identified antibiosis resistance to *D. radicum* in *B. spinescens* CA91061, showing a reduced number of pupae and high plant survival. Accessions of *B. spinescens* also show antibiosis resistance to the cabbage aphid *B. brassicae* (Singh et al. 1994), resistance to white rust *Albugo candida* [(Pers. ex. Lév.) Kuntze] (Peronosporales: Albuginaceae) and salt tolerance (Kirti et al. 1991), making it an interesting material for breeding. In the greenhouse we identified high levels of resistance to *D. radicum* in accessions *B. fruticulosa* PI663081 and *B. spinescens* BRA2994. These accessions also showed a high or moderate level of shoot and root vigour, and reduced fly dry weight. Both the higher insect mortality and the lower fly dry weight indicate that the resistance is based on antibiosis.

Next to the two highly resistant early-flowering species, moderately resistant material was found within the biennial species. In previous studies, high levels of antibiosis to *D. radicum* was found in *B. incana,* and moderate resistance in *B. macrocarpa* and *B. villosa*, using mainly plant performance parameters (Ellis et al. 1999). In our study of accessions of the same species (Table 3 and Online Resource 1), we found resistant candidates in all of these species in the field screen. *Brassica macrocarpa* BRA2944 and *B. villosa* BRA2922 lasted long before wilting, and had a relatively low proportion of wilted plants. *Brassica incana* BRA2856 also lasted long before wilting, but exhibited a high proportion of wilting plants. All individual plants of the two *B. incana* accessions produced flies in the greenhouse, though no larvae or pupae were collected from the same accessions in the field experiment. Plants with better general vigour have higher tolerance to insect attack (Painter 1951). Some plants with higher tolerance were able to regrow new roots from the undamaged part of the main root, thus it is likely that tolerance played a role in delaying wilting. The high number of flies and long period before wilting of *B. incana* BRA2856 could be due to low antibiosis, combined with a high level of tolerance to early larval feeding. Within-accession variation in some of these accessions was visible in the greenhouse, for instance in accessions where on some individual plants no root fly developed, while other plants showed a high percentage of eclosed flies (Table 3). Similarly, within-accession variation regarding cabbage aphid resistance has been reported by others as well (Ellis et al. 2000). Because accessions are heterogeneous, the within-accession variation may be genetic. From an accession with a high average fly survival, the plants that showed no root flies emerged may be resistant to *D. radicum*. Such individuals may definitely be interesting for further investigation. Selfings can be made and crosses within the accession and crosses with *B. oleracea* are suggested. The progenies should be screened for antibiosis to *D. radicum*.

In the field evaluation we tested a large number of accessions each with five plants and selected only accessions on which we found no *D. radicum*. Consequently, we have not selected resistant plants in heterogeneous accessions. In the greenhouse test the number of plants per accession was increased to 10. Also here heterogeneity was evident. Both the lack of correlations based on accession means between insect and plant traits, and the clear within-accession variation observed indicate that more attention should be paid to assessment of individual plants in these wild species related to *B. oleracea.*

Prospects for resistance breeding and use of genes

High levels of antibiosis resistance towards cabbage root flies were identified in two accessions, *B.*

spinescens BRA2994 and *B. fruticulosa* PI663081. They showed not only significantly reduced proportions of *D. radicum* eclosing, but also a significantly lower fly dry weight of survivors. These two accessions hold potential for studying the genetics of the resistance and for breeding of resistant cabbages. As genetic modification is not a marketable option in the current European context, it is important that the resistance mechanisms found in related species can be crossed into the cultivated *Brassica* genome. Both *B. fruticulosa and B. spinescens* (n = 8 and 16) have different chromosome number than *B. oleracea* (n = 9) (Ellis et al. 2000; Jensen et al. 2002), making the transfer of genes from *B. fruticulosa* and *B. spinescens* to *B. oleracea* problematic. To cope with that, advanced interspecific hybridisation techniques such as ovary, ovule and embryo culture (Takeshita et al. 1980; Diederichsen and Sacristan 1988; Bajaj et al. 1986) or protoplast fusion techniques (Kirti et al. 1991) could be helpful. Recently, interspecific hybridization between a *B. fruticulosa* and *B. oleracea* var. acephala has shown to be possible (Pelgrom et al. 2015), although fertility of the hybrid is an issue. The species *B. villosa, B. incana, B. cretica, B. insularis, B. macrocarpa, B. montana, B. rupestris, B. bourgeaui, B. hilarionis* and *B. drepanensis* belong to the *B. oleracea* complex (n = 9), and interspecific crosses with *B. oleracea* are possible (von Bothmer et al. 1995; Lazaro and Aguinagalde 1998; Faulkner et al. 1998). Among these species we found promising accessions in the field and medium levels of mean number of *D. radicum* in the greenhouse.

To apply the detected resistance in the practice of plant breeding when gene transfer by the afore mentioned methods is possible, might be by identifying resistance QTLs in intra-specific crosses between resistant and susceptible plants and to introgress these QTLs using marker-assisted selection. If gene transfer proves impossible, the homologs of the identified QTLs in *B. oleracea*, and the allelic variation of the putative causal genes in the *B. oleracea* complex can be investigated. Obviously, a reliable identification of resistant and susceptible parents within the donor species is needed and our work together with the entomological literature cited in the Introduction indicates that this is feasible. Assuming that introgression of the resistance trait into *B. oleracea* is successful, measures should be taken to avoid breakdown of the resistance, crop rotation being one of them. Applying integrated pest management can contribute to control the pest in an environmentally benign way. When more than one resistance gene is found, gene pyramiding may also increase durable resistance (Joshi and Nayak 2010; Li et al. 2014). Antixenosis resistance may also further strengthen antibiosis resistant and tolerant accessions.

Tolerance is a mechanism that supports plant survival and development to minimize fitness loss resulting from insect attack. Plants with high tolerance may be useful in a push–pull system, or in combination with integrated pest management. Yet tolerance supports the insect population to increase until tolerance breaks down. Obviously breeding for tolerance is not useful in controlling insect pests in the long run without incorporating other means of insect control. This is why we did not aim for selecting plants with high tolerance. Strong antibiosis resistance in combination with high tolerance is considered most desirable in our study.

In conclusion, we identified several accessions with medium to high levels of antibiosis resistance towards the cabbage root fly among wild *Brassica* species. Accessions with the highest levels of resistance belonging to the species *B. fruticulosa* and *B. spinescens* are difficult to cross with *B. oleracea*, but may be promising materials for studying the genetics of the resistance through a QTL mapping approach. Several other accessions (*B. villosa* BRA2922, *B. montana* BRA2950, *B. hilarionis* HRIGU12483, *B. macrocarpa* BRA2944) with medium level of antibiosis resistance and medium to high level of tolerance are more easily crossed with *B. oleracea*. As variation within accessions was observed, selection of the most resistant individuals within these accessions is important. In the greenhouse, using the proportion of eclosed flies is effective and efficient, thus this scoring method is highly recommended for root fly resistance screens.

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